A tale of two copies: evolutionary trajectories of moth pheromone receptors

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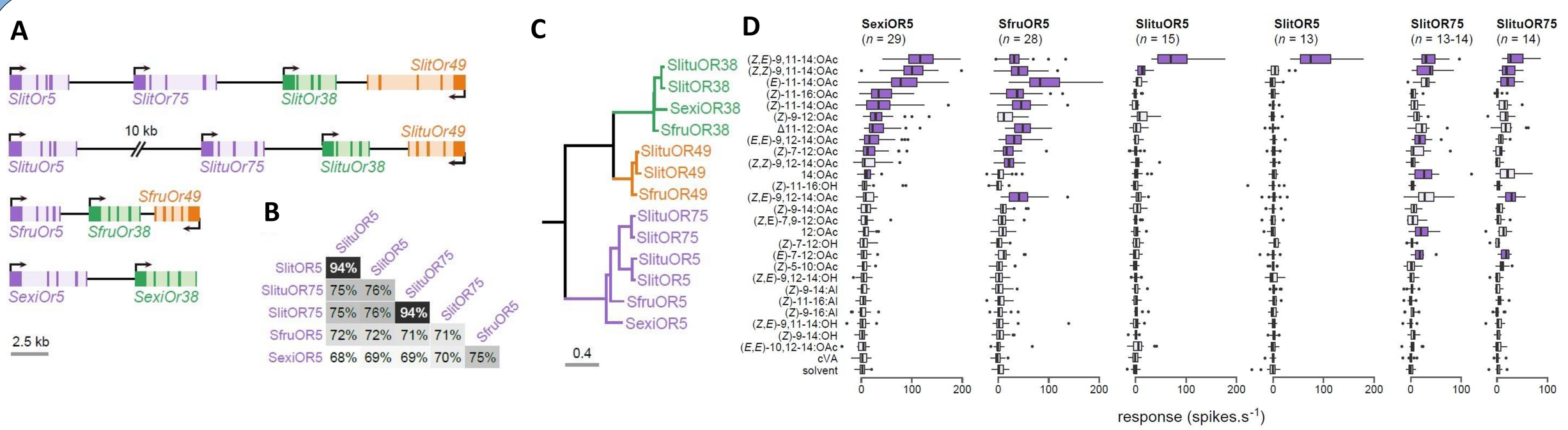
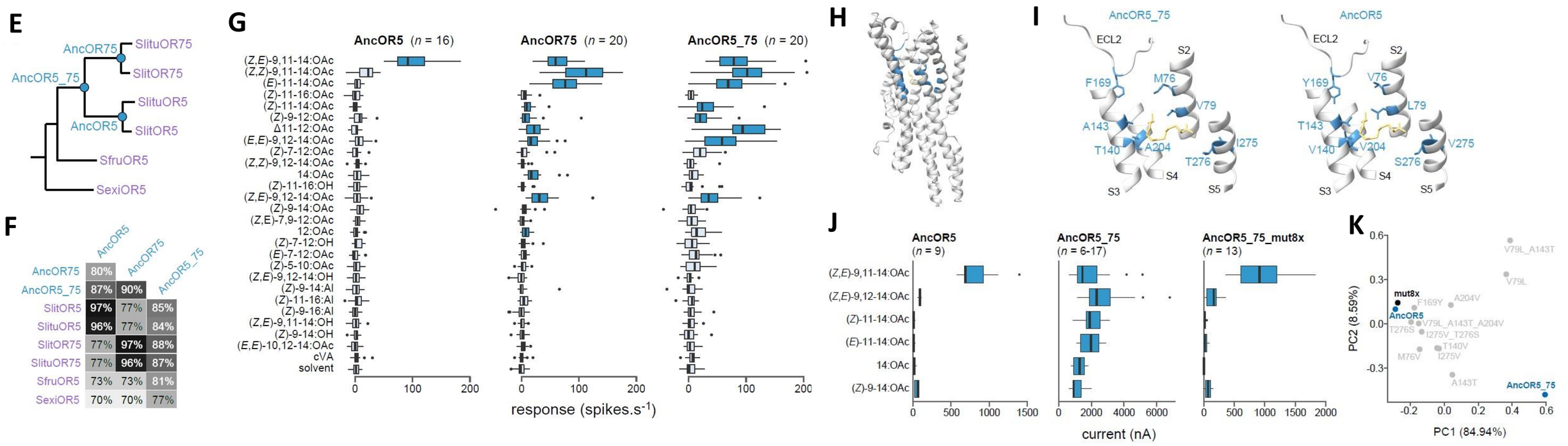
Introduction

Pheromone communication is an essential component of reproductive isolation in animals. *Spodoptera littoralis* has evolved its own repertoire of olfactory receptors (Ors) to satisfy its ecology, essentially through duplication events. Duplicates vary significantly in sequence identity and tuning breadths. However, how the duplicates diverged and acquired new functions is not known. Previous functional studies showed that SlitOR5 is specifically tuned to (*Z*,*E*)-9,11-14:OAc (Bastin-Héline *et al*., 2019), while SlitOR75, a recent duplicate of SlitOR5, can detect many compounds. Here, we used ancestral gene reconstruction using the codeml of the PAML suite and heterologous expression in drosophila neurons to resurrect AncOR5_75, their common ancestor, as well as AncOR5. Then, the 3D structure and binding pockets were predicted by AlphaFold2 and Deepsite, respectively. At last, ancestors were functionally characterized using *Xenopus* oocyte heterologous expression system, and site-directed mutagenesis experiments were performed on AncOR5_75 to recreate the evolutionary trajectory that led to SlitOR5 (Li & Capoduro *et al*., 2023).



(A) Synteny of Or5 orthologs and paralogs in the four species. (B) Identity matrix between amino-acid sequences encoded by *Or5* and *Or75* genes. (C) Phylogeny of ORs encoded by genes shown in (*A*). (D) Tuning breadth of OR5 orthologs and paralogs in *Spodoptera* species.



(E) Phylogeny of OR5 orthologs, paralogs and ancestors. (F) Amino-acid sequence identity. (G) Response spectra of *Or5*/*Or75* ancestors. (H) 3D structure of AncOR5_75. (I) Binding pockets of AncOR5_75 and AncOR5. (J) Response profiles of AncOR5, AncOR5_75 or AncOR5_75_mut8x. (K) Principal Component Analysis based on the responses of AncOR5, AncOR5_75 and its related mutants.

Conclusion

- Identified and functionally characterized six ORs from Spodoptera species that all clustered in the novel pheromone receptor clade.
- Evidenced a duplication of OR5 in a common ancestor of *S. littoralis* and *S. litura* and found that in these two species, one copy is also broadly tuned as AncOR5_75 while the other one is specific to (*Z*,*E*)-9,11-tetradecadienyl acetate.
- Identified eight amino acid positions in the binding pockets of these receptors whose evolution has been responsible for narrowing the response spectrum to a single ligand.

Scan me!

Acknowledgments

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Bibliography

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